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Effect of distillery spent wash on cytomorphological behaviour of sugarcane settlings

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Abstract: *Characteristics of crude and digested spent wash (distillery waste), and effect of their application on cyto-morphological attributes of settlings of eleven genotypes of sugarcane (Saccharum species hybrids) were studied. High concentrations of K, P, S, Fe, Mn, Zn and Cu contents and heavy metals were present in crude spent wash as compared to the digested one. Root meristem assay of settlings grown on crude and digested spent wash showed a detrimental effect on mitotic efficiency and also induction of de novo chromosomal aberrations viz. clump formation, chromosome stickiness, laggards and micronuclei formation etc. higher number of chromosomal abnormalities as compared to those of control conditions. Mitotic index of root-meristems of different genotypes showed a decline of 62.65 to 100% in crude spent wash treatment and 36.94 to 90.33% decline in digested spent wash treatments as compared to the control. However, an improvement to the extent of 27.73% was observed in the mitotic activity of root-meristems treated with diluted spent wash (1:5 v/V with water). Inhibitory effects of digested and crude spent wash were also visible on bud sprouting and settling height, but 1:5 aqueous dilution of spent wash stimulated the early growth attributes. Such beneficial effects of diluted spent wash were observed in most of the sugarcane genotypes.*

Key words: *Chromosome aberrations, Cytotoxicity, Distillery waste, Heavy metal, Pollution, Saccharum, Sugarcane*
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Introduction

Despite containing some heavy metals, the distillery spent wash is used as irrigation water for sugar cane crop as it contains all the essential elements required for the growth (Jain *et al*., 2004). In recent years, due to expansion of distilleries in the sugar cane growing countries, the indiscriminate disposal of spent wash in sugarcane cultivation lands adjacent to different industries is affected by heavy metal toxicity (Om *et al.,* 1994). Heavy-metal pollutants have a high bioaccumulation rate and they are slowly released in an organism, causing a number of damages. Ecogeochemical studies have shown that the highest level of heavy metals (Hg, Pb, Zn, Cu, Cd, Cr, Ni, V, As) was present in soils of territories of industrial enterprises (Taragkevieius, 1999).

The biological responses to an external hazardous agent that give a measure of exposure and that can be used to indicate harmful effects or to predict future harm are classified as biomarker (Earnst and Peterson, 1994). Assays of chromosome aberrations in plants are some of the oldest, simplest, most reliable, and least expensive biomarkers in the field of environmental mutagenesis (Constantin and Owens, 1982). Cytogenetic tests analyze the frequency and type of chromosome aberrations in mitotic cells because chromosomal disturbances may reflect a rapid response of organisms to the environmental toxicant exposure and can provide early warning signs of adverse long-term effects in the populations (Hose, 1994; Reno *et al*., 1994). An attempt has therefore been made in the present study to unearth the consequences of distillery spent wash as a source of irrigation water on *de novo* cyto-morphological changes in sugarcane.

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Materials and Methods

Eleven elite sugarcane genotypes (*Saccharum* species hybrids) of subtropical India *viz.* CoLk 9411, CoLk 91239, CoLk 9617, CoLk 9618, LG 94184, CoPant 9301, CoPant 93227, CoSe 96436, UP 9530, CoS 95255 and BO 91 were used in the experiment. Single bud setts (stalk cuttings having one bud each used for vegetative propagation) of each genotype were planted (bud-to-bud distance 10.0 cm) in large plastic trays containing farm soil and organic manure, with a pH of about 6-7 (commonly used in conventional sugarcane planting in sub-tropical India) and were subjected to different treatments of distillery spent wash: crude spent wash (BOD 40,000 mg l^{-1}), aqueous dilution of crude spent wash (1:5 v/V crude spent wash: water), digested spent wash (BOD 4,000 mg l^{-1}), and control (water treatment). The trays were kept under net house conditions (day temperature 31.1-37.6°C; night temperature 15.6-19.7°C) and periodic observations were taken with respect to bud sprouting and early growth of settling. Each treatment contained two replicates.

For nutrient analysis, spent wash was wet digested in nitric acid and perchloric acid (10:1) in 100 ml flasks on a temperature controlled hot plate inside fume chamber up to incipient drying stage as described in Jain *et al*. (2001) and Piper (1942). After cooling, the digest was made to volume with distilled water and filtered. In clear digest, nutrient content were determined using an atomic absorption spectrophotometer (AAS). Root tip assay was done to

find out the effect of spent wash on mitotic efficiency and chromosomal aberrations *de novo*. For cytological analysis, 1-1.5 cm long root tips from germinating setts were fixed directly in carnoy's 6:3:1 fluid (6 alcohol: 3 chloroform: 1 glacial acetic acid) as well as after pretreatment with 1:1 v/V aqueous solution of saturated para-dichlorobenzene and 0.002 M 8-Hydroxyquinoline for 3-3½ hr (Srivastava, 1995). The fixed root tips were transferred in 70% alcohol and stored at low temperature (10-12°C). The root-tips were hydrolyzed in 1N HCl for 30 minutes at 60°C, washed thoroughly and stained in 2% aceto-orcein for 5-6 hr. The stained root-tips were squashed on a glass slide in a drop of 45% glacial acetic acid. Well spread plates from at least 50 meristems per replication were scored for each treatment for various types of cytological effects. Mitotic Index (MI) was calculated using the following formula:

$$MI = \{A/ (A+ B)\} \times 100$$

where, A=number of dividing cells (metaphase and anaphase) and B= number of non-dividing cells. Changes in the MI were expressed as a factor of the mean MI from treated setts over the mean MI from control.

Presence and absence of chromosomal aberrations *e.g*. chromosome clumping, stickiness, cytomixis, grouping, and lagging *etc*. and their extent was recorded in all treatments. Data on bud sprouting were scored visually after one week of planting and graded as 0,1,2 and 3 based on nil, poor, average or excellent sprouting respectively. Settling height was recorded at 5 days' interval from 15 to 30 days after planting in all the treatments. All the data were recorded from five plants per genotype per treatment.

Results and Discussion

Nutrient analysis of spent wash showed that crude spent wash contained very high concentration of essential nutrients *viz.* K, P, S, Fe, Mn, Zn and Cu contents and heavy metals *viz.* cadmium, chromium, nickel and lead as compared to the digested one (Table 1). The pH of digested spent wash was alkaline (9.0) and crude spent wash was highly acidic (4.0) in nature. The emergence of roots from the setts treated with crude spent wash was very poor. The root growth was stunted and the root-tips were deformed in the crude spent wash treatment. The growth of roots was comparatively better in setts treated with digested spent wash; however the over-all root growth was affected badly. This was reflected by the reduced or hampered mitotic activity of root-meristem cells that could be judged by the mitotic indices of root meristems of different genotypes under various treatments (Table 2). The mean mitotic index was 1.3% in root meristems treated with crude spent wash, 5.35% in digested spent wash, 16.09% in spent wash: water (1:5) treatment and 14.51% in control. Mitotic efficiency of root-meristems of different genotypes showed a decline of 62.65 to 100% in crude spent wash treatment and 36.94 to 90.33% in digested spent wash treatment as compared to the control. However, a stimulatory effect to the extent of 27.73% was observed in the mitotic activity of root-meristems treated with 1:5 aqueous dilution of crude spent wash as compared to control (Table 2). The decrease in mitotic index indicated a

Table - 1: Presence of essential nutrients and heavy metals in crude and digested spent wash

Metals (mg ml^{-1} spent wash)	Digested spent wash (pH 9.0)	Crude spent wash (pH 4.0)
Essential nutrients		
P	35.1	77.6
S	765	1609
Fe	9.52	68.5
Mn	0.72	3.0
Zn	0.72	4.01
Cu	0.38	2.66
Heavy metals		
Cd	0.004	0.025
Cr	0.95	0.172
Ni	0.88	0.863
Pb	0.54	1.24

mitodepressive effect of spent wash treatment on cell division activities in root-tip cells of sugarcane. Similar mitodepressive response has been observed in *Allium cepa* root cells in response to insecticides, herbicides, pesticides and chemical mutagens treatment (Rao *et al*., 1987; Shanker *et al*., 1987; El-Khodary *et al*., 1989). Such a reduction in mitotic activity could be due to inhibition of DNA synthesis (Beu *et al*., 1976). The decrease in mitotic index indicates the loss of dividing cells, which may be attributed to the presence of heavy metals in spent wash, which interfere in the normal sequences of mitosis leading to disturbance of spindle function. Various chromosomal aberrations were present in high frequency in crude and digested spent wash treatment indicating their cytotoxic nature. The wide spectrum of cytotoxic effects induced by spent wash included spindle inhibition, chromosome stickiness, laggards, C-mitosis, delayed anaphases, multipolarity and bi/multi nucleate cells, chromosome fragmentation and micronuclei formation. Chromosomal aberrations were present only in crude and digested spent wash at metaphase as well as anaphase stages, however, these were almost absent in 1:5 aqueous dilution of spent wash treatment as well as control.

Inhibitory effects of digested and crude spent wash were also visible on bud sprouting and settling height (Table 3). However, effect of 1:5 aqueous dilution of crude spent wash was beneficial on early growth characteristics of settlings of most of the sugarcane genotypes. Growth of settlings treated with crude and digested spent wash was severely affected. Adverse effects of crude spent wash were higher as compared to the digested one. Settling height after 15 days of planting showed marked depression in crude and digested spent wash treatments (Table 3). This trend was continued when examined at 5 days intervals for early growth period of settling. This was in concurrence with the mitotic efficiency of the respective genotypes.

The mean mitotic index of sugarcane genotypes showed a decline of 91.62 and 62.45% in crude and digested spent wash treatments as compared to control, which indicated the inhibitory effect of spent wash on mitotic cell division of somatic cells. This, in

Table - 2: Mitotic efficiency of root-meristems of different sugarcane genotypes in response to distillery spent wash treatment

Genotype	Treatment						
	Crude spent wash		Digested spent wash		Crude Spent wash: Water (1:5 v/V)		Control
	MI %	% Δ	MI %	% Δ	MI %	% Δ	
CoLk 9411	0.45	-96.4	7.00	-36.94	14.60	+26.13	11.10
CoLk 91239	0.30	-97.7	7.33	-45.70	16.60	+22.96	13.50
CoLk 9617	0.60	-96.22	6.64	-58.19	18.00	+13.35	15.88
CoLk 9618	1.30	-91.15	5.10	-65.28	18.40	+25.42	14.69
LG 94184	0.80	-94.16	2.64	-81.02	14.10	+2.92	13.70
CoPant 9301	0.56	-96.47	3.66	-76.55	17.11	+7.61	15.90
CoPant 93227	1.50	-89.05	3.60	-74.26	17.50	+27.73	13.70
CoSe 96436	6.20	-62.65	9.30	-43.45	16.30	-1.81	16.60
UP 9530	1.60	-96.43	5.56	-66.91	16.22	-0.04	16.80
CoS 95255	0.00	-100.0	1.45	-90.33	14.23	-5.13	15.00
BO 91	1.60	-87.46	6.60	-48.28	13.93	+9.64	12.76
Mean	1.30	-91.62	5.35	-62.45	16.09	+11.79	14.51
Range	0.00 to 6.20	-62.65 to -100	1.45 to 9.30	-36.94 to-90.33	13.99 to 18.4	-1.81 to 27.73	11.10 to 16.80

MI % = Mitotic Index %; % Δ = % decrease or increase over the control, v = Volume of crude spent wash; V = Total volume of solution made up by water

Table - 3: Effect of distillery spent wash on bud sprouting and settling height (cm) of sugarcane genotypes

Genotype	Crude spent wash		Digested spent wash		Crude spent wash: Water (1:5 v/V)		Control	
	Bud* sprouting	Settling height**	Bud sprouting	Settling height	Bud sprouting	Settling height	Bud sprouting	Settling height
CoLk 9411	0	6.10	1	26.90	2	31.30	3	29.70
CoLk 91239	0	6.40	0	21.60	3	30.20	1	27.80
CoLk 9617	0	7.20	1	20.60	3	38.18	3	23.10
CoLk 9618	1	4.60	1	16.20	3	43.80	2	30.00
LG 94184	0	1.80	1	6.80	3	33.30	2	24.50
CoPant 9301	0	2.20	1	20.20	2	49.20	3	32.10
CoPant 93227	1	4.80	1	10.50	3	35.00	2	24.80
CoSe 96436	0	27.8	1	31.90	2	37.10	3	36.90
UP 9530	0	10.20	1	21.30	2	31.90	3	28.80
CoS 95255	0	0.00	0	2.40	2	45.80	3	34.80
BO 91	0	14.50	1	18.50	2	26.10	2	20.70
Mean	0.18	7.78	0.81	17.90	2.45	36.53	2.27	28.47
Range	0 - 1	0.0- 27.8	0 - 1	2.4 - 31.9	2 - 3	26.1 - 49.2	1 - 3	20.7 - 36.9

* = Visual scoring of bud sprouting after one week of planting; 0-Nil, 1-Poor, 2-Average, 3-Excellent

** = Settling height measured after 30 days of planting

turn affected the early growth of settlings as reflected by bud emergence and shoot height of settlings. The correlation coefficients of mitotic indices with settling height raised under crude and digested spent wash were highly positive (mean correlation coefficient r = 0.94 and 0.90 respectively) at all stages of experiments (Table 4). Similarly bud sprouting was highly correlated with settling height (mean r = 0.97) and mitotic index (mean r=0.99) (Table 5). Adverse effects of tannery effluents and wastes of distillery and sugar mill on growth of plants have been observed in several crops. The Sakari (sulphitation) sugar mill effluent decreased the mitotic index and percentage of the mitotic phases in the treated roots of *Allium cepa* (Kumar, 1999). The distillery effluent elicited the significant deleterious effect on seed germination and early seedling growth in pigeon pea (Karande and Ghanvat, 1994). The tannery effluent concentration at 75 and 100% (v/V in water) killed the plants of *Oryza sativa*, *Acacia holosericea* and *Leucaena leucocephala*, however, the leaf area and biomass of plants treated with 25% tannery effluent showed an increase over the control (Arunyal *et al*., 1994). 10% concentration of distillery effluent was found favourable for seed germination in forest seedbeds compared to ordinary water (Pandey and Soni, 1994). In experiments conducted in the major sugarcane growing areas in Guanxi, China, inhibitory effects of vinasse (liquid waste or dunder or spent wash) were observed on emergence of seed cane (Li *et al*., 2007). Most seed-cane buds could not sprout probably due to the anaerobic conditions produced by application of vinasse due to its high BOD and COD. In the present study also, dilution of spent wash (1:5 v/V with water) showed marked improvement (11.79%; mean value) in mitotic index over control along with the improvement in the early growth characteristics (bud sprouting, root emergence and settling height). The spent wash used in the present study contained very high concentrations of K, P, S, Fe, Mn, Zn and Cu contents and heavy

Table - 4: Correlation coefficients between mitotic index and settling height of sugarcane genotypes under crude and digested spent wash treatments at different time intervals

	Crude spent wash	Digested spent wash
Settling height at 15 DAP*	0.97	0.87
Settling height at 20 DAP	0.96	0.91
Settling height at 25 DAP	0.93	0.91
Settling height at 30 DAP	0.91	0.91
Mean	0.94	0.90

*DAP = days after planting

Table - 5: Correlation coefficients of bud sprouting with mitotic index and settling height of sugarcane genotypes

	Bud sprouting
Mitotic index	0.99
Settling height	0.97

metals such as cadmium (0.025 mg ml^{-1}), chromium (0.172 mg ml^{-1}), nickel (0.863 mg ml^{-1}) and lead (1.24 mg ml^{-1}). It is known that heavy metals can cause oxidative DNA damage (Hartwig, 1995) leading to the induction of DNA strand breaks, DNA-protein cross-links and formation of chromosomal aberrations. Further, compounds of Cr, Ni, Co, Cd and Fe are genotoxic to organisms (Hartwig, 1995). Due to disturbance in the synthesis of proteins and nucleic acids, nucleoprotein configuration of chromosomes is changed causing spindle inhibition. The somatic variation generated *de novo*, which when channeled into formation of vegetative shoots may bring about bud-sport mutations in sugarcane, although the presence of multiple genomes bestows buffering capacity to the palaeopolyploids /evolutionary polyploids to withstand large-scale gain/loss or alteration in their genetic material (Lavania *et al*., 2006).

The present study showed that the rate of cell division (measured in terms of active mitotic index) decreased in crude and digested spent wash treatments. It is therefore concluded that the use of spent wash as such not only upset the normal rate of cell division but also induced various chromosomal aberrations that might lead to mutation in the affected organism. However, use of diluted spent wash promoted mitotic cell division leading to improved early growth attributes in most of the sugarcane genotypes.

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